Measuring Shape Changes in Active Membrane Proteins that Correlate to Their Function

NIST scientists are using a unique side-by-side approach, combining FTIR and optical spectrometers to understand how the shapes of membrane proteins change as they perform their function. Using a membrane-bound proton pump, bacteriorhodopsin (BR), as a mode,l new understanding of how to measure structure-function relationships in these important proteins is emerging.

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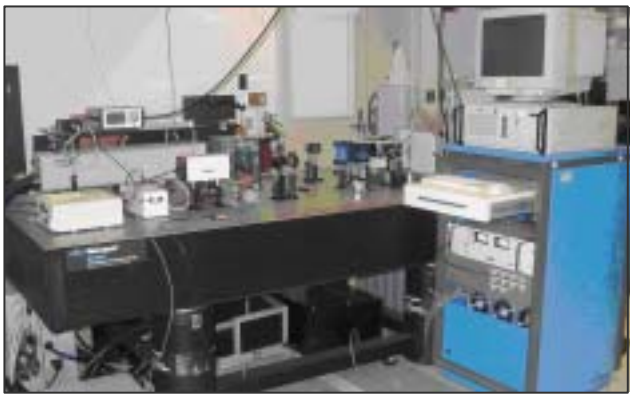
There is an growing awareness of the importance of active membrane proteins and the need to greatly expand studies in this area. For example, the NIH roadmap for future research includes significant funding for the isolation, crystallization, and study of membrane proteins. Most drugs used today are directed at altering the function of membrane proteins such as G protein-coupled receptors (GPCR). Unfortunately, it is difficult to obtain dynamic structural information about membrane proteins through the traditional x-ray crystallography approach. New methods are needed. Before committing large expenditures of money and effort, there should be a quality control procedure to establish that any crystallized preparation shows the same kinetic behavior as in the membrane. Furthermore, based on published studies (RWH), there are steps that can be taken either to prevent a deterioration of normal function or to reverse those which have occurred.

The best protein crystals are made in procedures which employ detergent. In published studies of one of us (RWH), performed with the active membrane proton pump bacteriorhodopsin (BR), it was demonstrated that the briefest exposure of a membrane protein system to the most dilute detergent dramatically alters specific membrane lipid-amino acid associations and the normal kinetics of the system. A new method is being developed. The ultimate goal is to relate conformational changes of a protein to its function, which for BR is the electrogenic transfer of protons across the membrane

NIST is developing unique measurement tools that elucidates the structure-function relationship of membrane-bound protein drug receptors. A significant impact of the ability of drug developers to design new therapeutic molecules is expected.

With a crystal known to function in its proper mode, we can apply mathematical approaches based on linear algebra that can deconvolute the overlapping optical spectra, in a time-resolved study, into the individual pure states that make up the kinetic sequence. Even when a crystal of a protein is found to follow a perturbed kinetic sequence we can isolate kinetic states which are similar to those of the native protein for comparison. As a first step in developing standardization procedures that can be applied to membrane protein crystals, we are planning parallel time-resolved FTIR and optical studies on the same sample. In addition to proper lipid protein interactions, native behavior requires a certain minimum amount of hydration. In a crystal, this is in the form of the water of crystallization. Using our side-by-side FTIR and optical spectrometers, we can determine the minimum number of water molecules (using the strong IR vibrational mode for H_2O) required for normal kinetics (determined optically)..

In FY2006, we were able to localize the precise steps in the kinetic sequence of the laser pulse-initiated photocycle of BR, where most of the membrane potential is generated by the proton current. We also, in collaboration with Paul Smith (NIH), designed and built special optics and supports for measuring the kinetics of the BR photocycle on the standard 1 inch barium fluoride allowing us to use a single sample for both the optical and IR spectrometers.



Time-resolved multichannel optical data are collected by a unique spectrophotometric system. This second-generation 96-channel spectrophotometer has: higher resolution (16-bits), higher maximum sampling rate (200 kHz), independent programmable gain and offset for each analog channel, logarithmic as well as linear scheduling of samples, and higher system reliability. This state-of-the-art optical system allows a complete photocycle of the protein to be obtained with each laser pulse.

Future plans (near and long term)

1. To perfect the step scan capability of our FTIR spectrometer to allow the obtaining of clean time-resolved spectra at both 100 and 200 KHz.
2. To try to determine the minimum ratio of H_2O molecules to BR that allows normal photocycle behavior.
3. To determine if profound changes in photocycle behavior under different conditions of pH and temperature are due to changes in the mosaic or raft organization of membrane lipids resulting in different membrane-protein interactions.
4. To build or participate in the building of a new instrument that uses microscope optics combined with fiber optics and an intensified charge coupled device detector that enables the obtaining of optical and IR spectra on single crystals of membrane protein.
5. To obtain time-resolved X-ray diffraction data on crystals previously standardized by our combined optical and FTIR spectroscopic analyses.

Publications:

Richard W. Hendler, John W. Kakareka, Paul D. Smith, Thomas J. Pohida, and Curtis W. Meuse. **"Proton-pumping Capabilities of the M-fast and M-slow Photocycles of Bacteriorhodopsin"** Submitted to *Journal of Physical Chemistry B.*

Richard W. Hendler, Richard I. Shrager, and Curtis W. Meuse. **"The Ability of Actinic Light to modify the Bacteriorhodopsin Photocycle Revisited: Heterogeneity vs. Phototcooperativity"** Ready for submission to *Journal of Physical Chemistry B.*